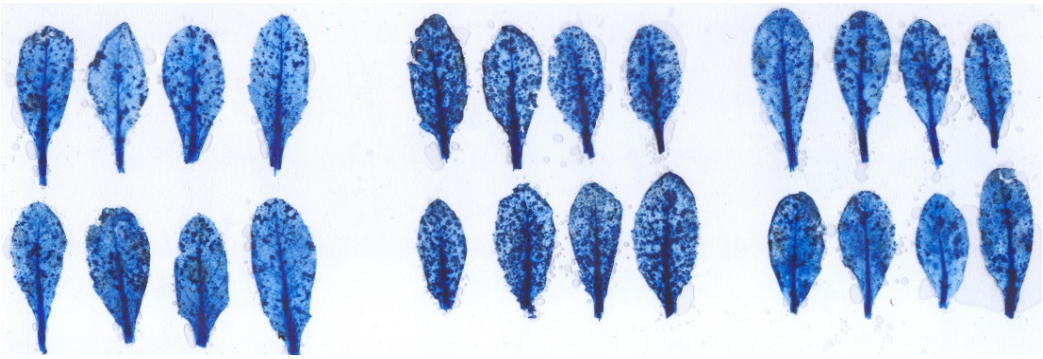
A

Col-0 *AtrbohD-3* *AtrbohF-3*

B



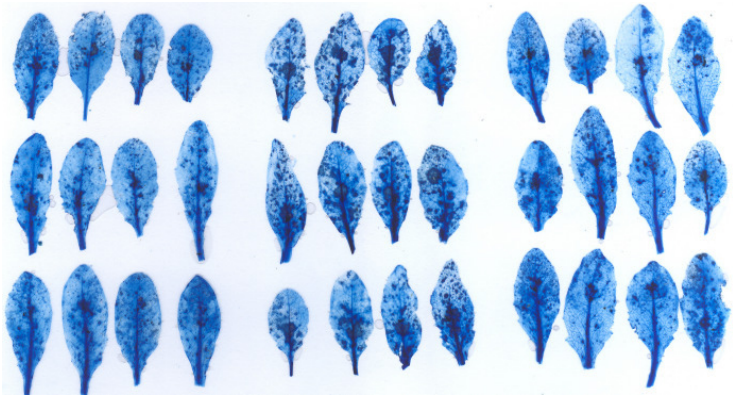
Col-0	*AtrbohD-3*	*AtrbohF-3*
0.19 ± 0.0149 [a]	0.42 ± 0.0155 [b]	0.19 ± 0.0144 [a]

Supplemental Figure S1. Necrotic symptoms of *A. brassicicola* infection on an independent set of Arabidopsis mutants (Torres et al., 2002, 2005). Mutant lines *AtrbohD-3* and *AtrbohF-3* are impaired in *rbohD* (At5g47910) or *rbohF* (At1g64060) mRNA expression. A, Whole plants showing symptoms of the fungal infection 9 d after inoculation. Plants were sprayed with a conidial suspension containing 10^6 *A. brassicicola* spores in 1 mL distilled water. The experiment was repeated twice with similar results. B, Trypan blue staining for the detection and quantification of cell death in wild type (Col-0), *AtrbohD-3* and *AtrbohF-3* leaves 9 d after inoculation with *A. brassicicola*. Results are presented as necrotised leaf area compared to the total surface of leaf blades analysed by Adobe Photoshop (1 is equal to100 % leaf surface), and they represent means ± SE of 15 Arabidopsis leaves per genotype. The experiment was repeated twice. Different letters indicate statistically significant differences between genotypes using Tukey's post-hoc test for pairwise comparisons ($\alpha = 0.01$).

A

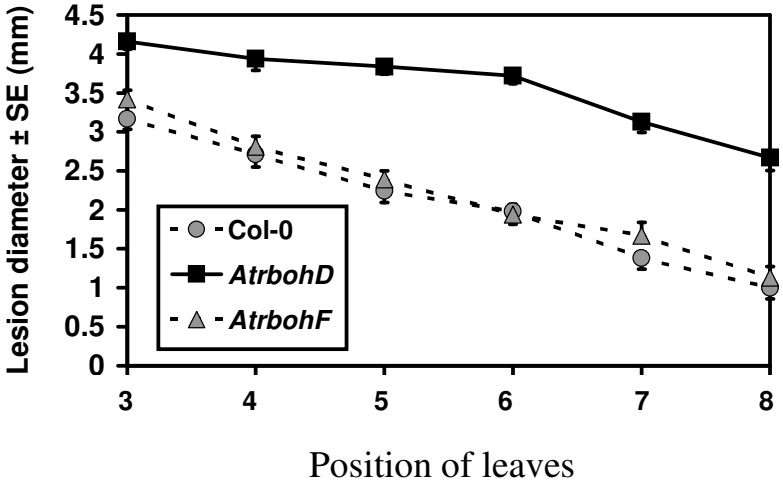
Col-0 *AtrbohD* *AtrbohF*

B

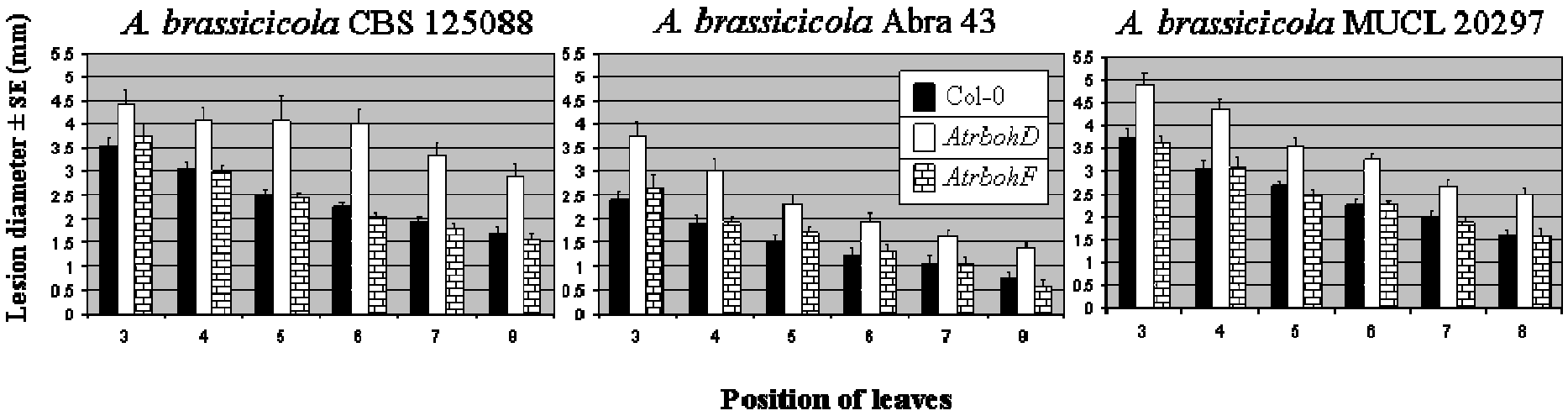


Col-0 *AtrbohD* *AtrbohF*

C



Supplemental Figure S2. Evaluation of *A. brassicicola* drop-infection on wild type (Col-0), *AtrbohD* and *AtrbohF* Arabidopsis plants (9 d after inoculation). A, Symptoms of *A. brassicicola* drop-inoculation (5 x 10^5 conidia in 1 ml distilled water) on whole plants. Ten µl droplets of conidial suspension were transfered to leaves of 25 Arabidopsis plants. Cell death is accelerated in *AtrbohD* T-DNA insertion line (SALK_070610). B, Trypan blue staining of drop-inoculated leaves exhibits that *A. brassicicola*-induced lesions are larger on leaves of *AtrbohD* plants. C, Quantification of fungal-induced lesion diameters on six true leaves between positions 3 and 8. Lesions were measured on 15 to 18 leaves (one lesion per leaf) for each data point.



Supplemental Figure S3. Evaluation of necrotic symptoms caused by 3 different *A. brassicicola* strains on wildtype, *AtrbohD* and *AtrbohF* Arabidopsis genotypes 9 d after inoculation. Six consequtive true leaves between positions 3 and 8 were drop-inoculated with 10 µl conidial suspension droplets (5 x 10^5 conidia in 1 ml distilled water). Each data point represents the mean of lesion diameters measured on 8 to 10 plants. *AtrbohD* plants consistently develop larger lesions regardles of the *A. brassicicola* strain plants were inoculated with. *A. brassicicola* strain CBS 125088 (used throughout this work) and strain MUCL 20297 exhibit similar symptoms on the 3 Arabidopsis genotypes while lesion diameters after inoculation with strain Abra 43 are consistently reduced.

Supplemental Figure S4. Leaf age of wild type (Col-0) Arabidopsis plants significantly affects success of fungal colonisation by *A. brassicicola* strain CBS 125088. Oldest true leaves in positions 1 to 4 contain higher fungal biomass levels than the rest of the leaves in higher positions 9 d after inoculation. Leaves were drop-inoculated and leaf discs (5 mm in diameter) centred on the lesion were excised by using a cork borer. Discs collected from six plants composed a biological sample and *A. brassicicola* biomass levels were determined from leaf total DNA extracts by real-time PCR. Three independent biological samples were analysed in triplicates. Data points represent the ratio between the qPCR signals of the *A. brassicicola*-specific ribosomal DNA ITS region and the Arabidopsis-specific gene *At4g26410*. Different letters indicate statistically significant differences using Tukey's post-hoc test for the statistical analysis ($\alpha = 0.01$).